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Effect of the hormone brassinolide and polymer hydrogel on anatomical characteristics of okra (*Abelmoschus esculentus* L.) under two different watering periods

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This study was conducted in a private field in Basrah AL-Qurna for the growing season 2022-2023 to study the effect of the hormone brassinolide, hydrogel polymer and two different watering periods on some anatomical characteristics of okra leaves. The experiment included a study of the effect of three factors: (BR) at two concentrations (3, 6) mg/l, (SAP) at two concentrations (50, 100) g/ m² soil, and two watering periods (3, 6) days. The experiment was designed depening on the complete random block design (R.C.B.D.) with three replicates for each treatment, and the least significant difference test was (0.05). The results showed that the treatment of plants with the (BR) led to a significant increase in all the studied characters, as the (SAP) had a significant effect on all the studied traits, and the binary interactions significantly affected all the studied featuers. In conclusion, significant increase occurred in all studied traits at a hormone concentration of 6 mg/ l and a polymer at a concentration of 100 g/ m² soil and an irrigation period of 3 days compared to the control treatment and an irrigation period of 6 days.

Keywords: Okra plant, Brassinolide hormone (BR), Hydrogel polymer (SAP), Irrigation periods.

INTRODUCTION

Okra, Abelmoschus esculentus L., is one of the important summer vegetable crops in the world. It belongs to the Malvaceae family. It is cultivated in large areas in Asia and Africa (Singh and Nigam, 2023). It spread widely in the world's tropical. It is an annual herbaceous plant with a main root from which dense branches branch out, and a cylindrical, branching green stem with a length of about 45-180 cm. The leaves are longnecked, palmate, and heart-shaped, the flowers are bisexual, actinomorphic, and the lower parts are hypogynous. The flowers are formed gradually from the base of the stem towards the apex, it is clustered yellow (Ouis, 2016), and their fruits are green cylindrical capsule divided from the outside by long protrusions containing a large number of seeds 10-100 seeds that are spherical or oval in shape, shiny and soft to the touch, with a gray color. Its nutritional importance is in terms of containing carbohydrates, proteins, some salts, and antioxidants, thus protecting the body from cancer, diabetes and respiratory diseases. Its leaves are also used as animal fodder. Furthermore, okra fruits have insoluble fibers that work on the integrity of the intestine and colon. The fruits havean important to cholesterol level in the blood (Jones, 2017). Drought is one of the of the important proplems that whorl suffers from naw, , . Among the means that can be used to increase the plant's tolerance to drought is using compounds such as the Super Absorption Polymers (SAP) type. The BR has a role in reducing water stress. Alenazi (2011) found that spraying the okra plant with the (BR) caused an increase in the average thickness of the upper and lower epidermis cells thickness of the columnar and spongy layer, and the thickness of the wood and bark. Given in view of the importance of the increacead demand for it ,and in order to the improve the plants resistan to drought stress condaition, this experiment was conducted, to find out the effect of some soil-enhancing polymers and the role of the brassinolide hormone in reducing this strees and improving production.

MATERIALS AND METHODS

The experiment during the agricultural season 2022-2023 in

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one of the civil fields in the north of Basra district, using a non-heidden plastic house with dimensions of 50 x 9 m and an area of 450 square meters using the drip irrigation method took samples Random from different places of plastic house soil before agriculture deeply 0 - 30 cm for the purpose of conducting chemical and physical analyses of these samples at the Center for the Sea Sciences- Basrah University (Table 1).

Table 1. Some of the chemical and physical properties of the soil and water of the field.

Adjective	Value
E.C des m	2.05
pH	8.02
Total N (mg kg)	13.40
Available P (mg kg)	2.62
Available K (mg kg)	2.63
OM %	1.46
Separates of Soil (%)	
Sand	36.00
Silt	21.00
Clay	43.00
Soil texture	Sandy loam
Irrigation Water	
E.C des m	1.83
pH	8.46

Then the drip irrigation system was installed, and the treatments were distributed randomly to the experimental units according to the Randomized Complete Block Design (RCBD) according to the split-split plot design, as the irrigation periods represent the main plots. While the polymer treatments counted the sub-plots, the improved polymer was added and dispersed under the soil at a depth of 15-20 cm with two concentrations (50 or 100) g/ m² soil, and then the soil was moistened. A solution of the BR was prepared with two concentrations (3 or 6) mg/ l. Experimental measurements were taken from three plants in each experimental unit at the end of the agricultural season. The anatomical sections were prepared using the paraffin wax technique according to the method mentioned in Jones (2017). The average thickness of the upper and lower epidermis with the cuticle, the columnar.

RESULTS AND DISCUSSION

Effect of the (BR) and (SAP) on the anatomical characteristics of the leaf and stem under two different irrigation periods: 1- Average epidermis thickness with cuticle (micrometer): Table 2 showed that the highest average thickness of the upper epidermis with cuticle was which gave the lowest percentage of grain moisture amounted to 8.11%. The difference of varieties in the percentage of moisture in grain may be attributed to the chemical composition of grain and the degree of its hardness, in addition to genetic factors and extent of their effect on the moisture content of grain. Alternately, the cause could be related to the hygroscopic properties of wheat grain and the fact that, during harvest, environmental factors like humidity and temperature have a significant impact on moisture Table 2, Effect of brassinolide hormone, polymer hydrogel on the average thickness of upper epidermis with cuticle (micrometer) content. These findings supported the information provided by regarding the interaction, Table 2's findings revealed that there were

Table 2. Effect of BR, SAP on the average thickness of upper epidermis with cuticle (micrometer).

watering periods (day)	Polymer concentration g m² soil	Hormone concentration mg l			Interaction polymer with irrigation
		0	3	6	
3 days	0	2.08	2.50	2.08	2.22
	50	3.33	3.33	3.33	3.33
	100	2.91	3.33	4.00	3.41
6 days	0	1.67	2.08	2.50	2.08
	50	3.33	2.50	2.08	2.53
	100	3.33	3.33		3.47
BR × ir					average effect of irrigation
	3	2.77	3.05	3.13	2.99
	6	2.77	2.63	2.77	2.73
average effect of BR		2.77	2.84	2.96	
BR × SAP					average effect of SAP
	0	1.87	2.27	2.29	2.15
	50	3.33	2.99	2.70	2.98
	100	3.12	3.33	3.87	3.44

$LSD_{0.05}$

BR	SAP	Ir	RB × SAP	BR × ir	SAP × ir	BR × ir × SAP
0.42	0.47	0.62	0.73	0.58	0.62	1.01

significant differences in the percentage of grain moisture, with the combination of the Babylon cultivar and) first date producing the highest average moisture content of 10.53% (without significantly differing from the combination of the Wafia cultivar and first date, which produced an average of 10.13%), and the combination of the Ibaa 99 cultivar and second date producing the lowest average for this trait of 7.43% recorded in the treatment. The concentration of the BR was 6 mg/l, the SAP was 100 g/ m² soil, and an irrigation period of 3 days, as the average was 4.00µm, with a significant difference with the comparison treatment when the plants were irrigated for 6 days, with a value of 1.66 µm. While the treatments (BR with 6 mg/l, SAP concentration 100 g/ m² soil, SAP concentration 6 mg/ l, irrigation period 3 days, polymer concentration 100 g/ m² soil, and 6 days watering) gave similar values for thickness as shown in Table 2.

Table 3. Effect of brassinolide hormone, polymer hydrogel on the thickness of lower epidermis with a micrometer cuticle under two different irrigation periods.

watering periods (day)	SAP g m² soil	RA mg/l			SAP × r with ir
		0	3	6	
3 days	0	2.50	2.92	2.08	2.50
	50	2.92	3.75	2.92	3.19
	100	3.33	2.92	3.96	3.40
6 days	0	1.67	2.08	1.67	1.81
	50	2.50	2.50	2.71	2.57
	100	2.92	2.92	3.75	3.19
RA × irr					Irrigation
	3	2.92	3.19	2.99	3.03
	6	2.36	2.50	2.71	2.52
RA		2.64	2.85	2.85	
RA × SAP					SA P
	0	2.08	2.50	1.88	2.15
	50	2.71	3.12	2.81	2.88
	100	3.13	2.92	3.85	3.30

$LSD_{0.05}$

RA	SAP	IR	SAP × RA	RA × Ir	SAP × Ir	SAP×IR × RA
0.55	0.74	2.01	1.01	1.54	1.55	1.72

Table 4. Effect of BR, SAPon the thickness ocolumnar layer under two different irrigation periods (micrometer).

watering periods (day)	Polymer concentration g m² soil	Hormone concentration mg l			Interaction polymer with irrigation
		0	3	6	
3 days	0	7.50	7.92	7.92	8.50
	50	9.58	10.83	9.17	8.89
	100	9.17	7.92	12.08	9.72
6 days	0	6.87	7.50	7.50	7.99
	50	8.75	9.58	8.33	8.19
	100	8.33	7.50	9.17	8.33
BR × ir					average effect of irrigation
	3	8.75	8.89	9.72	9.12
	6	7.99	8.19	8.33	8.17
Average BR		8.37	8.54	9.03	
SAP × BR					average SAP
	0	7.19	7.71	7.71	7.53
	50	9.17	10.21	8.75	9.38
	100	8.75	7.17	10.52	9.03

$LSD_{0.05}$

Hormone	Polymer	irrigation	hormone × Polymer	hormone × irrigation	Polymer × irrigation	hormone polymer × irrigation ×
1.18	0.88	1.38	1.82	1.52	1.24	2.58

The thickness of the lower epidermis with the cuticle (micrometer): The case was almost identical to the thickness of the upper epidermis, the results of Table 3 that thickness of the lower epidermis was recorded in the treatment, the concentration of the BR was 6 mg l, the SAP concentration was 100 g m² soil, and an irrigation period of 3 days, as the average was 3.96 µm with a significant difference with the comparison treatment when the plants were irrigated for 6 days, with a value of 1.67 µm. Also, the two-way interactions recorded close values in the thickness of the lower epidermis and at a 3-day irrigation period. 3- Thickness of the columnar layer of the medium tissue of the leaf (micrometer): It is clear from the results shown in Table 4 that the highest thickness of the columnar layer was recorded in the plants treated with the BR in 6 mg/ l and treated with SAP in 100 g/ m² soil and 3 days watering, with an average thickness of 12.08 µm and with the control plants, as it recorded was 6.87 µm at watering of 6 days. The bilateral interactions also notes a significant superiority; similar values were recorded, as shown in Table 4.

Table 5. Effect of BR, SAP on average thickness of spongy tissue layer of leaf is µm.

Watering periods (day)	SAP g/m² soil	BR concentration mg/ l			SAP × irrigation
		0	3	6	
3 days	0	7.29	7.50	7.50	7.43
	50	8.75	10.42	9.58	9.58
	100	7.92	10.42	11.67	10.00
6 days	0	6.87	6.25	7.08	6.74
	50	7.50	7.92	9.17	8.19
	100	7.50	8.75	9.58	8.61
RB × ir					average effect of ir
	3	7.99	9.44	9.58	9.00
	6	7.29	7.64	8.61	7.85
average RB		7.64	8.54	9.10	
SAP × BR					average effect of SAP
	0	7.08	6.88	7.29	7.08
	50	8.12	9.17	9.38	8.89
	100	7.71	9.58	10.62	9.31

$LSD_{0.05}$

BR	SAP	Ir	SAP × BR	BR × ir	SAP × irr	BR × ir × SAP
0.77	0.88	0.79	1.33	0.97	1.07	1.81

Table 6. Effect of BRe, SAP on the thickness of the wood under two different watering periods (micrometer) .

watering periods (day)	SAPconcentration g/ m² soil	BR concentration mg/ l			Interaction polymer with irrigation
		0	3	6	
3 days	0	07.08	12.08	11.67	10.28
	50	17.92	15.83	19.17	17.64
	100	24.17	24.75	28.92	25.94
6 days	0	9.17	12.50	15.00	12.22
	50	14.17	17.50	21.25	17.64
	100	20.00	22.50	25.42	22.64
BR × irn					average effect of irrigation
	3	16.39	17.56	19.92	17.95
	6	14.44	17.50	20.56	17.50
average BR		15.42	17.53	20.24	
BR × SAP					average SAP
	0	8.12	12.29	13.33	11.25
	50	16.04	16.64	20.21	17.64
	100	22.08	23.62	27.17	24.29

$_{0.05}LSD$

BR	SAP	ir	BR × SAP	BR × ir	SAP × ir	BR×irn × SAP
2.33	2.47	5.52	3.91	4.26	4.34	5.92

4- Thickness of spongy layer of the middle tissue of the leaf (micrometer): The results in Table 5 show that the plants treated with the BR at in 0.6 mg/ l and theSAP at 100 g/ m² soil were significantly superior, as they recorded the highest average thickness of 11.67 µm at an irrigation period of 3 days, compared to the lowest thickness of 6.87 µm at the control plants and irrigated in 6 days. represents a comparison treatment anatomical changes of the leaf as a result of the effect of the BR and the SAP at different concentrations b concentration 6 mg l tropolymer 100 g/ m² soil 3 days and treatment C concentration 6 mg/ l tropolymer 50 g/ m² soil and a period of 3 days and treatment d represents the BR 0 and SAP 50 g/ m² soil and an irrigation period of 3 days and e represents a BR 6 mg/ l, SAP 0 and watering 3 day and f of 0 BR and SAP 50 g/ m² soil with an watering of 6 days and of 0m g/l of BR and SAP50 g/ m² soil with watering of 3 days. Tested under X400 power.

5- The thickness of the Xylem layer in the steem is micrometer: The results of Table 6 and Fig. (3 and 4) show that the plants treated with the BR at with of 6 mg l and the SAP at a withof 100 g m² soil at watering of 3 days showed a significant superiority as they recorded the highest thickness rate of 28.92 µm compared to the lowest thickness rate recorded in the control plants, was 7.08 µm. The two interactions showed similar values and significantly affected the average wood thickness.

Table 7. Effect of BR andSAP on Phloen area under two different irrigation periods (micrometer).

watering periods(day)	SAPg/ m² soil	BR concentration mg/l			SAP × irrigation
		0	3	6	
3 days	0	7.92	8.75	8.75	8.74
	50	17.50	14.17	17.92	16.53
	100	22.50	23.33	29.17	25.00
6 days	0	10.00	10.00	12.50	10.83
	50	15.00	15.00	20.83	16.94
	100	22.50	21.67	23.75	22.64
BR × irrigation					average effect of ir
	3	15.92	15.42	18.61	16.67
	6	16.25	15.56	19.03	16.81
average effect of BR			15.99	18.82	
BR × SAP					average SAP
	0	8.96	9.38	10.63	9.65
	50	16.25	14.58	19.37	16.74
	100	22.50	22.50	26.46	23.82

$_{0.05}$LSD

BR	SAP	Irrigation	BR × SAP	irr × BR	SAP × ir	BR × SAP × ir
2.29	3.29	7.04	4.36	5.33	5.58	6. 72

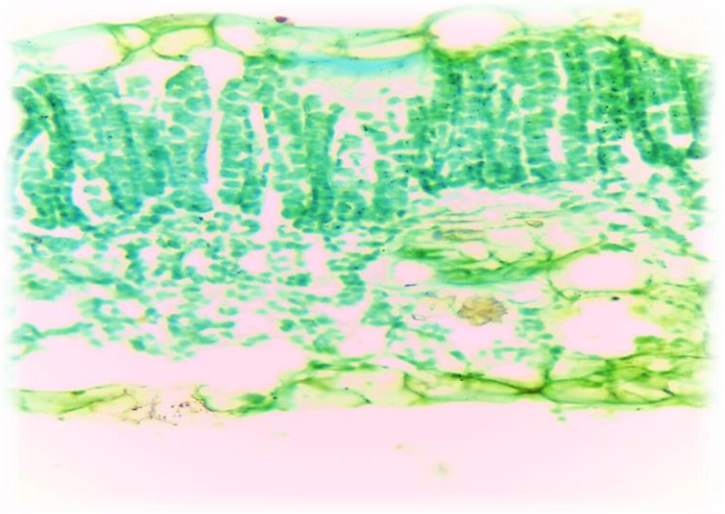

Figure 1. **okra leaf represents a comparison treatment**

6 - Phloen thickness (micrometer) The results of Table 7 show that the plants treated with the BR with 6 mg/ l, SAP with 100 g/ m² soil and a 3-day watering were significantly superior, as they recorded the highest thickness rate of 29.17 µm, compared to the control plants irrigated for 6 days, as they recorded the lowest thickness rate of 7.92 µm at a 3-day watering. Also, the two-way watering periods (day) SAPconcentration g/ m² soil BR concentration mg/ l InteractionSAP a significant effect, especially during the 3-day irrigation period, and similar values were recorded.

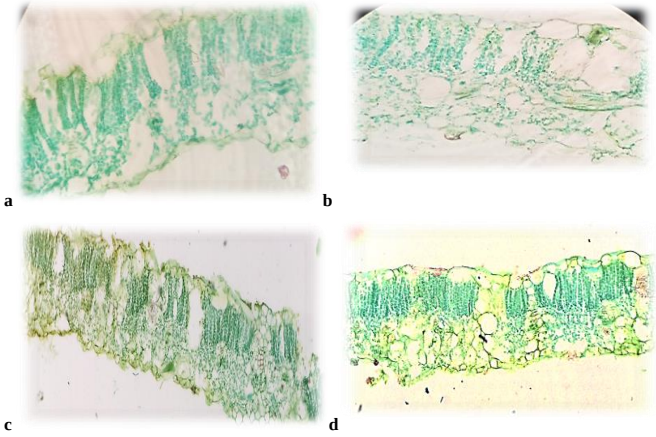

Figure 2. The anatomical changes of the leaf as a result of the effect of the BR and the SAP at different concentrations a, b concentration 6 mg l tropolymer 100 g/ m² soil 3,6 days and treatment C concentration and d control period of 3,6 days and treatment d represents control Tested under X400 power.

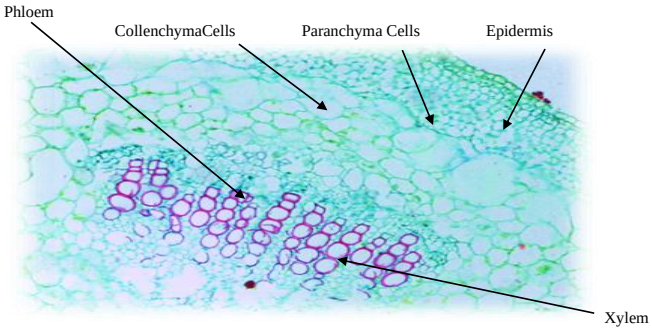

Figure 3. represents the okra steem.

DISCUSSION

The results of the current study showed the significant excellence of spraying with the BR and the use of a SAP in improving the anatomical characteristics and reducing the effect of water stress on the leaf and stem tissues of the okra plant, as shown in Tables (2- 7).

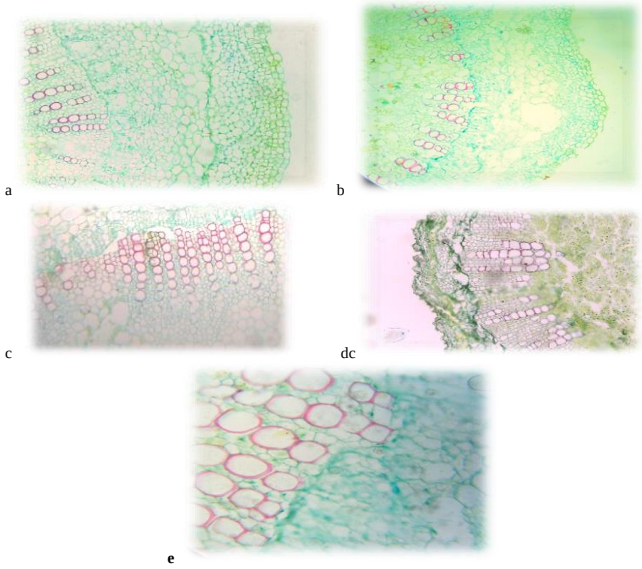

Figure 4. a and b control 3, 6 day, c represents the BR concentration treatment 6 mg l and SAPconcentration 100 g m² soil and 6 day irrigation period, d represents the BR in 6 mg l and SAP 100 g/ m² soil with 3 day wateing, e represents the BR zero and the SAP 100 g/ m² soil and 3 days irrigation, and e represents the BR concentration treatment 6 mg/ l and the SAP was zero for the same period and was tested under powers of 100X, except for f under powers of 400X.

All the factors of the study had a significant effect on the characteristics of the thickness of the cuticle with the upper epidermis and the lower epidermis, the thickness of the columnar layer and the spongy layer in the leaf, the 16 thickness of the xylem and phloen of the stem of the okra plant. As the plants sprayed with the BR excelled significantly in all anatomical characteristics, and those indicators increased at a concentration of 6 mg/ l ,took to the role of this BR in stimulating cells and helping to divide and elongate by activating the genes responsible for the formation of RNA in the cell chromosomes and the occurrence of a sudden change in the thickness of the cells as a result of the increase in content vegetative and flowering, improving the process of photosynthesis and increasing the metabolic outputs, which reflected positively on the increase of sugars and proteins in the leaves and their accumulation in the plant body, thus increasing the vegetative growth and increasing the thickness of plant cells and tissues (Alnajjar and Alapresam, 2018 ; Alwan, 2016). The division and elongation of cells are due to the role of the BR in stimulating and increasing cell division in parenchymal cells (AlMaleki, 2022), as shown in Figures (1 and 2). The effect of the BR in increasing the thickness of the epidermal layer, thickness of basal layer, spongy layer, the

increase in thickness of the wood vessels, phloem, and the elongation of the wood vessel, as shown in Table 6 and Figures (3 and 4), which caused an increase in the efficiency of the transport and delivery process, the storage of water and nutrients, and the accumulation of metabolites and salts, which leads to changes Histological and anatomical cracks in the vascular region and the cortex, and the accumulation of nutrients in the pulp region, and this agrees with what was stated by (Alnajjar *et al*., 2021). The current study proved that the SAP had a role in improving the anatomical characteristics, especially the high concentrations of it; it gave the best results in all studied characteristics, especially the concentration 17 of 100 g / m² soil, as shown in Tables (2- 7). The absorption of mineral elements led to an increase in the formation and construction of green pigments, chlorophyll, activity the efficiency of photosynthesis, and a lot of the formation of carbohydrates, reflected positively in the vegetative and anatomical characteristics. The results from Tables (2- 7) showed that the two irrigation periods had a significant effect on some anatomical features and that the 3-day period gave the best average thickness of the upper epidermis with the cuticle and the lower epidermis with the cuticle. The best thickness of the epidermis and the spongy layer of the middle tissue in the leaf, while the 6-day period was the best in the thickness of the wood and bark stem as shown in Figures (1-4), as theincrease in cell resistance and tolerance to stress was evident in the treatments that were sprayed with the BR. These results are consistent with what he mentioned by Olaranont *et al*. (2020); the SAP also showed a clear role in mitigating the effect of stress and improving anatomical characteristics as a result of improving the vegetative and physiological characteristics of plants, providing soil moisture content and improving water use efficiency (Almaleki , 2022).

Irrigation periods have significantly affected anatomical traits, their development, and plant response to adaptation to different conditions (Sonto *et al*., 2022). Water stress reduces the rate of vegetative growth, decreases the effectiveness of photosynthesis, decreases the growth of roots, and thus decreases the flow rate of water and nutrients to the vegetative system (Al-shammari *et al*., 2020). The effect of the double and triple interactions is attributed to the study factors' positive effect on the okra plant's anatomical characteristics

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All authors read and approved the final manuscript.

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